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Award Number: W81XWH-04-1-0831

TITLE: Prognostic Value of Allelic Imbalance in Prostate Biopsy

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REPORT DATE: September 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-07-2005	Final	1 Jan 2004 – 30 Jun 2005

4. TITLE AND SUBTITLE
Prognostic Value of Allelic Imbalance in Prostate Biopsy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0831

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of New Mexico
Albuquerque, NM 87131

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Purpose: The novel Concept that is being tested in this project is that allelic imbalance (AI) in tissue obtained at prostate biopsy can serve as a sensitive and independent marker for staging and predicting disease recurrence in prostate cancer. Scope: The two Aims of the project are to(i)determine whether the number of sites of AI in biopsy predicts pathological staging following prostatectomy and (ii) to determine the independence, positive and negative predictive values, sensitivity of AI in biopsy tissues as a prognostic marker for prostate cancer. Major Findings: The number of sites of AI has been measured in DNA from 110 prostatectomy tissues, 83 tumor adjacent, histologically normal prostate tissues, 43 biopsy specimens with a positive diagnosis of prostate cancer, and 22 specimens of normal prostate tissue from men without disease. The results demonstrate that AI is increased (i)in tumor and tumor-adjacent prostate tissues relative to normal prostate tissues, (ii) as a function of Gleason Sum and (iii)in recurrent prostate tumors. Significance: Allelic imbalance in prostate cancer tissues may provide a novel diagnostic and prognostic marker.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	10	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION

Autopsy studies demonstrate that 10-20 million men in the United States have undiagnosed prostate cancer, indicating that the vast majority of men with prostate cancer are asymptomatic throughout their lifetimes. However, the introduction of current screening modalities for prostate cancer, particularly the measurement of prostate-specific antigen (PSA) levels, have greatly increased the number of men referred for prostate biopsies and subsequently diagnosed with invasive prostate cancer. The choices of definitive therapy for most men with clinically localized disease are usually radical prostatectomy (RP) and radiation therapy (RT). The consequences and complications of these treatments are frequently severe, including incontinence, urethral stricture, impotence and death. Between 30 and 40% of men with clinically localized tumors choose RP accepting the associated morbidity and mortality because it offers the chance for cure. However, about 40% of clinically localized tumors treated with RP are subsequently determined extraprostatic on pathological examination, and may not be curable by RP alone. Thus, men with extraprostatic disease often suffer the morbidity of RP without curative benefit. Similarly, because current prognostic markers in prostate biopsy tissue cannot reliably differentiate tumors that will remain indolent from those that will rapidly progress, men receive RT or RP and experience their associated morbidity and mortality although, in many cases, the treatment is unnecessary and management with "watchful waiting" would be more appropriate. **Therefore, it is imperative to identify informative prognostic markers of prostate cancer progression that differentiate at the time of biopsy those men who will benefit from RT or RP from those who can be spared their expense, consequences, risks and reduced quality of life.**

We have shown previously that the content of telomere DNA ("TC", a surrogate marker for telomere length) is an independent predictor of disease-free survival in prostate cancer. We hypothesize that (i) critically shortened telomeres generate genomic instability and thus, phenotypic variability in neoplastic prostate tissues, and (ii) this promotes the genesis of lethal, metastatic tumor cells. ***A highly significant prediction of this hypothesis, and the focus of this proposal, is that allelic imbalance (a reflection of genomic instability) in prostate biopsy tissue predicts staging and future disease recurrence.*** To test this proposition, we have developed a multiplex, PCR-based method for assessing allelic imbalance (AI), a measure of genome instability, at sixteen non-linked microsatellite loci in the genome. The method uses commercially available primers, reagents, instrumentation and analysis software, and is suitable for analysis of fresh, frozen or paraffin-embedded archival tissues. The AI assay requires only 1-2 ng of DNA, does not require paired normal tissue, can be performed on tissue containing mixtures of tumor and normal cells, and therefore is particularly well-suited for prognostic assessment of prostatic biopsies.

BODY

Tasks: The agreed upon specific aims and tasks to be completed under the Hypothesis Award are as follows:

Aim One: *To determine whether the number of sites of allelic imbalance (AI) in biopsy predicts pathological staging following prostatectomy.* This will be accomplished by assessing the association between AI in prostate biopsy with the pathological stage of the patients' paired prostatectomy specimen. The tasks associated with this aim are:

Task 1: Purify DNA from approximately 300 archival specimens of prostate biopsy and prostatectomy tissues.

Task 2: Measure AI in DNA from the 300 archival specimens of prostate biopsy and prostatectomy tissues.

Task 3: Determine the association between AI in prostate biopsy with the pathological stage of the paired prostatectomy specimen.

Aim Two: *To determine the independence, positive and negative predictive values, sensitivity (i.e. frequency of false-positives) and specificity (i.e. frequency of false-negatives) of AI in biopsy tissues as a prognostic marker for prostate cancer.* This will be accomplished by associating the number of sites of AI in prostate biopsy with patients' recurrence data. The task associated with this aim is:

Task 4: Using data from Task 2, determine the association between AI in prostate biopsy and patient outcome.

Progress Relative to Tasks:

Task 1: Purify DNA from approximately 300 fresh and archival specimens of prostate biopsy and prostatectomy tissues.

We have purified DNA from the following 258 archival prostate tissues:

- 110 prostatectomy tissues
- 83 tumor adjacent, histologically normal prostate tissues
- 43 biopsy specimens with a positive diagnosis of prostate cancer
- 22 specimens of normal prostate tissue from men without disease

Task 2: Measure AI in DNA from the 300 archival specimens of prostate biopsy and prostatectomy tissues.

We have measured AI in all of the 258 specimens listed above.

Task 3: Determine the association between AI in prostate biopsy with the pathological stage of the paired prostatectomy specimen.

Most of the archival biopsy samples that we have been able to collect and successfully analyze did not have patient-paired prostatectomy tissue available, and vice versa. Therefore, we first analyzed AI as a function of tissue source (normal, tumor adjacent or tumor). As shown in Figure 1, the 2-sided nonparametric Kruskal-Wallis Test indicates a highly significant increase in the mean number of sites of AI in tumor adjacent or tumor tissue relative to normal prostate tissues ($p<0.0001$). Similarly, the mean number of sites of AI in prostate biopsies containing tumor tissue was actually higher than the mean number of sites in unmatched prostatectomy specimens.

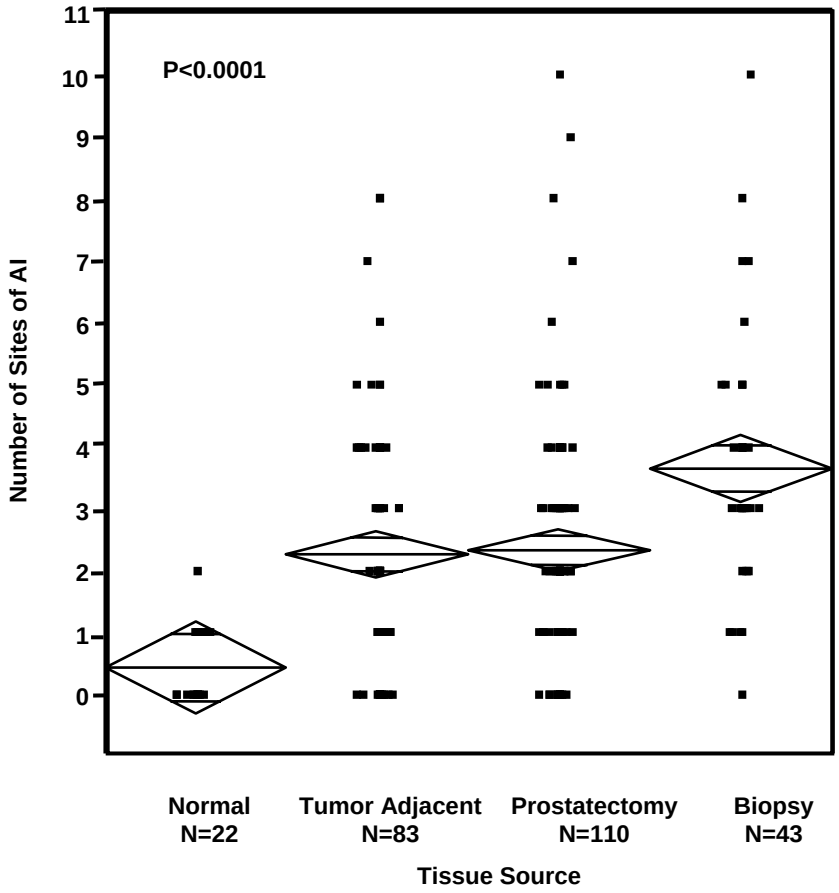

Figure 1. Numbers of sites of allelic imbalance (AI) in normal, tumor-adjacent, prostatectomy and biopsy tissues. The number of sites of AI is shown on the y-axis. The sources of the tissues and number of samples in each group (N) are shown on the x-axis. The line across each diamond represents the group mean. The height of each diamond represents the 95% confidence interval for each group. Statistical significance (p) was determined using the 2-sided nonparametric Kruskal-Wallis test. Although the data points are horizontally shifted, some are still overlapping, and therefore not visible. See text for additional details.

We next evaluated the association between AI and Gleason Sum. Tumor tissues were stratified by the Gleason Sum as follows: Group A: Gleason Sum 3-6 (N=33), Group B: Gleason Sum 7 (N=53), Group C: Gleason Sum 8-9 (N=11). As shown by nonparametric Wilcoxon Rank Sums test (Figure 2A), there was a highly significant association between the mean number of sites of AI and Gleason Sum (p=0.003). A similar result was obtained when the data were analyzed by logistic regression (Figure 2B), which again demonstrated a highly significant association between AI and Gleason Sum (p=0.001).

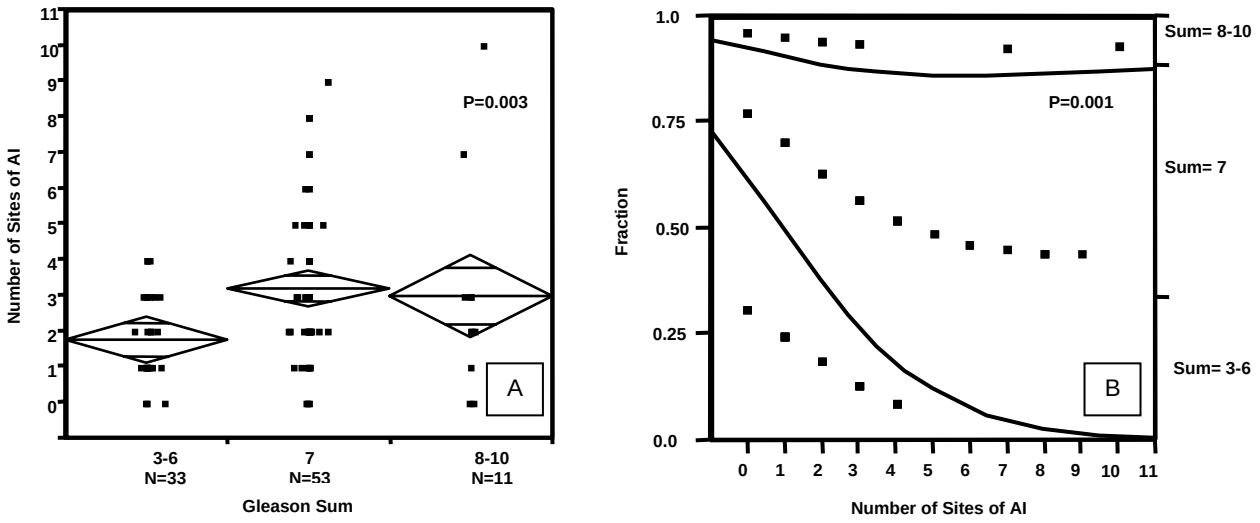

Figure 2. Numbers of sites of allelic imbalance (AI) in tumor tissues stratified by Gleason Sum. Tumors were stratified into three groups (3-6, 7, 8-10) by Gleason Sum. Panel A: The number of sites of AI are shown on the y-axis. The Gleason Sum group and the number of samples in each group (N) is shown on the y-axis. The line across each diamond represents the group mean. The height of each diamond represents the 95% confidence interval for each group. Statistical significance (p) was determined using the 2-sided nonparametric Kruskal-Wallis test. Although the data points are horizontally shifted, some are still overlapping, and therefore not visible. Panel B: The relationship between AI and Gleason Sum was evaluated by logistic regression. The number of sites of AI is shown on the x-axis. The fraction of tumors with Gleason Sums of 3-6, 7 or 8-10 are shown on the y-axis. See text for additional details.

In summary, these data indicate that: (i) the number of sites of AI in tumors, biopsies and tumor adjacent prostate tissues is significantly greater than the number of sites in normal tissues, demonstrating potential diagnostic value, (ii) the number of sites of AI in biopsy tissue is at least as great, and likely greater, than the number of sites in prostatectomy tissues and (iii) the number of sites of AI is associated with Gleason Sum. ***Taken together, the results are consistent with the conclusion that AI in biopsy tissues predicts Gleason Sum in tumors.***

Task 4: Using data from Task 2, determine the association between AI in prostate biopsy and patient outcome.

At least 5 years of follow up data was available for 85 men in the cohort. Recurrence was defined as distant metastasis, biochemical recurrence, i.e. rising PSA, or death as a consequence of prostate cancer. Nonparametric Wilcoxon Rank Sums test (Figure 3) demonstrates a significant association between AI and recurrence (p=0.04).

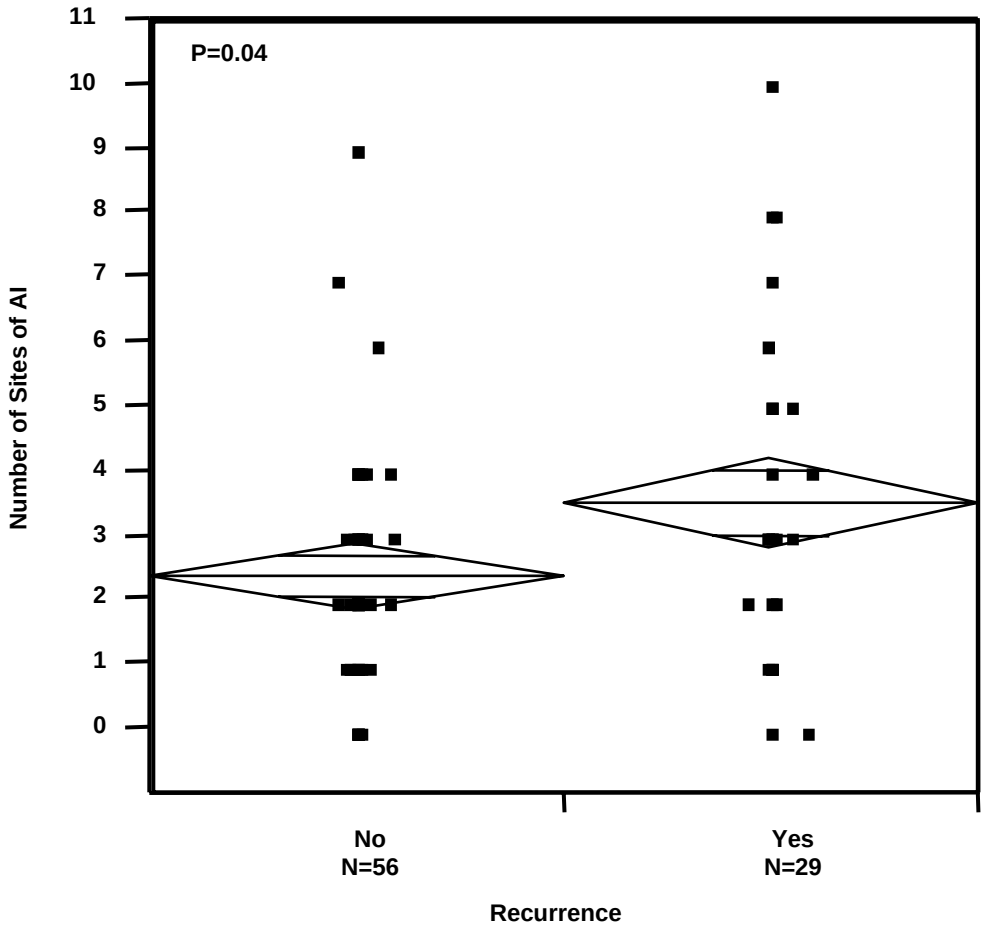

Figure 3. Numbers of sites of allelic imbalance (AI) in tumor tissues stratified by prostate cancer recurrence. Tumors were stratified by recurrence. The number of sites of AI is shown on the y-axis. Recurrence status and the number of specimens in each group (N) are shown on the x-axis.The line across each diamond represents the group mean. The height of each diamond represents the 95% confidence interval for each group. Statistical significance (p) was determined using the 2-sided nonparametric Kruskal-Wallis test. Although the data points are horizontally shifted, some are still overlapping, and therefore not visible. See text for additional details.

PLANS FOR CURRENT YEAR

We have obtained approximately 90 pairs of patient matched tumor and biopsy tissues with associated recurrence data. We will extract DNA from these tissues in the current year and analyze AI and its association with recurrence as described above.

KEY RESEARCH ACCOMPLISHMENTS

- Purified and quantitated genomic DNA from 258 prostate tissues.
- Measured extent of allelic imbalance (AI) in 258 prostate tissues.
- Demonstrated that AI is increased in tumor and tumor-adjacent prostate tissues.
- Demonstrated that AI increases as a function of Gleason Sum.
- Demonstrated that AI is increased in recurrent prostate tumors..

REPORTABLE OUTCOMES

- A database has been produced that contains anonymous patient histories, including age at diagnosis, treatments, Gleason Sum, tumor stage, pelvic node and seminal vesicle involvement, grade, size, length of disease free survival or date and cause of death and diagnosis.
- These data were presented at the 2004 AACR Symposium, "Telomeres, Telomerase and Cancer" in San Francisco, California, the 2005 FASEB Experimental Biology Meeting in San Diego, California and the 2005 Roswell Park Memorial Institute Conference, Prostate Cancer: Roadmap to the Future, in Niagra Falls, New York.
- One paper concerning breast cancer biology that utilized methods of AI measurement developed under this grant has been submitted for publication and it is anticipated that an additional manuscript will be submitted within the next six months.
- A patent disclosure has been filed for the use of the allelic imbalance assay in cancer diagnosis and prognosis.

CONCLUSIONS

- Allelic imbalance in prostate cancer tissues may provide a novel diagnostic and prognostic marker.

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